REMARKS/ARGUMENTS

Claims 77-96, presented hereby in place of claims 29-76, are pending. Support for the presented claims is found in the original claims and in the specification, as detailed below.

Claims 29-76 are cancelled, hereby, without prejudice or disclaimer.

Due to the election of species requirement, claims 29-44, 49-57, 60-64 and 66-73 were examined.

As explained in detail, below, the present claims are presented to resolve the issues raised in the rejections of record under §112, ¶1. Additionally, the present claims take into consideration expertise gained in the prosecution of the counterpart application before the European Patent Office (EPO), which led to recent allowance by the EPO of the main claim, i.e.:

> A method for characterizing samples having particles, by monitoring fluctuating intensities of radiation emitted, scattered and/or reflected by said particles in at least one measurement volume, the monitoring being performed by at least one detection means, said method comprising the steps of:
> a) measuring in a repetitive mode a number of photon counts per time interval of defined length,
> b) determining a distribution function of the number of photon counts per said time interval,
> c) determining a distribution function of specific brightness of said particles on basis of said distribution function of the number of photon counts by finding out the model of the sample yielding the closest fit between the experimentally determined and an expected distribution function of the number of photon counts, wherein the expected distribution function of the number of photon counts is determined using characteristics of the spatial brightness function of the equipment
> – employing values of volumes of sections of the measurement volume corresponding to a selected set of values of the spatial brightness and considering the volumes as variables depending on modeling parameters of the spatial brightness function and

- determining the values of these parameters which yield the closest fit between an experimentally determined and a calculated distribution of the number of photon counts.

The claims examined, pursuant to the requirement for election of species, were rejected under 35 USC 112, ¶1, for allegedly lacking descriptive support in the application as originally filed (a *new-matter* rejection) and under 35 USC 112, ¶1, for allegedly lacking enablement. Reconsideration of the rejections under §112, ¶1, is requested in view of the replacement claims submitted, hereby, and the remarks, as follow.

Applicant submits that claims 77-96, presented hereby, fully satisfy the written-description and enablement requirements of §112, ¶1. Applicant provides the following comparison between the present claims and the subject application disclosure to show that the presently claimed subject matter is, both, (i) described and (ii) enabled in accordance with the subject application as originally filed.

(i) Written Description Requirement

"The test for sufficiency of support in a parent application is whether the disclosure of the application 'reasonably conveys to the artisan that the inventor had possession at the time of the later claimed subject matter.'" *Vas-Cath Inc. V. Mahukar*, 19 USPQ2d 1111, 1116 (Fed. Cir. 1991). *In re Kaslow*, 217 USPQ 1089, 1096 (Fed. Cir. 1983). Presence or absence of literal support is not the issue. *Ex parte Harvey*, 3 USPQ2d 1626 (Bd. Pat. App. & Inter. 1987). To comply with the written description requirement the specification need not describe the claimed invention *in ipsis verbis*." *In re Edwards*, 196 USPQ 465 (CCPA 1978).

> In order to comply with the written description requirement, the specification "need not describe the claimed subject matter in exactly the same terms as used in the claims; it must simply indicate to persons skilled in the art that as of the [filing] date the applicant had invented what is now claimed." [*Citations omitted.*] . . . [T]he failure of the specification to specifically mention a limitation that later appears in the claims is not a fatal one when one skilled in the art would recognize upon reading the specification that the new language reflects what the specification shows has been invented.

All Dental Prodx LLC v. Advantage Dental Products Inc., 64 USPQ2d 1945, 1948 (Fed. Cir. 2002).

By way of illustration, features (limitations) of the present claims are matched with corresponding *description* in the subject-application disclosure, as follows:

Method step: *"measuring by detection means, in a repetitive mode, a number of photon counts per time interval of defined length"*

According to the paragraph bridging pages 7 and 8 of the specification, the number of detected photons is counted preferably many times, repeating the procedure in a series of preferably consecutive time intervals, in order to obtain statistically meaningful data. The length of the time interval is the duration of the time interval during which the number of photon counts is determined. The length of the time interval is expressed in microseconds or milliseconds or other units of time. According to the specification, page 7, last complete paragraph, appropriate detection means include, e.g., an avalanche photo-diode and conventional photo-diodes, as well as photomultipliers. In Example 1 of the specification, for instance, a mixture of rhodamine dyes is analyzed. In an experiment underlying application Figure 1, the time interval is 10 μsec and the measurements are performed repetitively over a total data collection time of 50 sec. In an experiment underlying

application Figure 2, the time interval is 40 μsec and the measurements are performed repetitively over a total data collection time of 50 sec.

Method step: "determining an experimental distribution function of the number of photon counts measured per time interval"

At page 7, first complete paragraph, the specification discloses that one should determine the number of times a certain number of photon counts has been obtained. The distribution is a function of the number of photon counts, expressing either the relative or absolute number of observed events when a particular number of photon counts has been obtained. As explained in specification Example 1, Figure 1 shows distributions of the number of photon counts experimentally determined at time intervals of 10 μsec over a data collection time of 50 sec; Figure 2 shows a distribution of the number of photon counts experimentally determined for a solution of rhodamine 6G at time intervals of 40 μsec over a data collection time of 50 sec; and Figure 5 shows the distribution of the number of photon counts experimentally determined from three samples (rhodamine 6G, tetramethylrhodamine, and a mixture of these two dyes) at time intervals of 40 μsec over a data collection time of 50 sec.

Method step: "determining a distribution function of specific brightness of the particles based on the experimental distribution function of the number of photon counts measured"

The distribution functions of specific brightnesses of particles from three samples (rhodamine 6G, tetramethyrhodamine, and a mixture of these two dyes) are graphically represented in application Figure 6. These distribution functions were determined based on the distribution function of the number of photon counts measured (i.e., measurements recorded in application Figure 5). The peak

in the distribution function of specific brightness of rhodamine 6G is situated at about 108 kHz/molecule; whereas, the peak of tetramethylrhodamine is centered at about 37 kHz/molecule. The distribution function of specific brightness corresponding to the mixture of these two dyes has two peaks centered, indeed, near the values obtained for the two dyes, separately.

Method step: *"by fitting an expected distribution function of the number of photon counts against the experimental distribution function of photon counts, wherein the expected distribution function of the number of photon counts is calculated using characteristics of a spatial brightness function"*

The specification discloses (pages 12 and 13) the methods of least squares fitting, ITR, ITC and, ITRC as exemplary alternatives for selecting the model yielding a fit between experimentally determined and expected theoretical functions. Application Figure 7 illustrates, exemplarily, this method step corresponding to the measurement of the mixture of rhodamine 6G and tetramethylrhodamine.

In the middle graph of Figure 7, a model of the sample assuming two different molecule species present in the sample was applied. Fitting between the experimentally determined and the theoretically expected functions on basis of this model shows homogeneously distributed residuals that indicate a close fit.

In contrast to the middle graph, in the lower graph of Figure 7, a model of the sample assuming merely one molecule species present in the sample was applied. The fit curve is obviously apart from the experimental one, i.e., there is no close fit between the experimentally determined and the expected distribution functions of the number of photon counts.

As explained on page 5 of the specification, the presently claimed invention provides a method for calculating the expected distribution of the number of photon counts. The presently claimed invention teaches the importance of giving adequate consideration to the interaction between the spatial brightness function characteristic and the optical set-up (page 6, first complete paragraph). Values of volumes of the sections of the measurement volume corresponding to a set of values of spatial brightness can be employed as characteristics of the spatial brightness function when determining the expected distribution of the number of photon counts. This is disclosed in detail on pages 15 to 16 of the specification. Alternatively, mathematical expressions are employed, as disclosed at page 16, first complete paragraph. With respect to, e.g., the aforesaid experiment on the solution of rhodamine 6G, the characteristics of the spatial brightness function where used in the calculation of the expected distribution function of the number of photon counts (specification page 21).

Method step: *"employing values of volumes of sections of the measurement volume corresponding to a selected set of values of the spatial brightness function and considering the volumes as variables depending on modeling parameters of the spatial brightness function and selecting the values of these modeling parameters which yield the closest fit between the experimentally determined and the expected distribution of the number of photon counts"*

Values of volumes of the sections of the measurement volume corresponding to a set of values of spatial brightness can be employed as characteristics of the spatial brightness function when determining the expected distribution of the number of photon counts. This is disclosed in detail at pages 15 to 16 of the specification. With respect to, e.g., the aforesaid experiment on the solution of rhodamine 6G, the characteristics of the spatial brightness function where used in the

calculation of the expected distribution function of the number of photon counts (specification page 21).

(ii) Enablement Requirement

Satisfaction of enablement under 35 U.S.C. 112, first paragraph,

> requires nothing more than objective enablement. . . . [A] specification . . . *must* be taken as complying with . . . 35 U.S.C. 112 *unless* there is reason to do doubt the objective truth of statements relied upon therein.

Staehelin v. Secher, 24 USPQ2d 1513, 1516 (BPA & I 1992) (*emphasis in original*). In order to sustain a rejection for lack of enablement under §112, first paragraph, and shift the burden to a patent applicant, the PTO must cite evidence in support of any allegations of non-enablement, in addition to explaining *why* it doubts the truth of statements of enablement made in the specification. *In re Sichert*, 196 USPQ 209 (CCPA 1977).

Lack of enablement under §112 is not established by mere allegations of undue breadth, that is, by merely arguing that claims read on non-disclosed embodiments. *Horton v. Stevens*, 7 USPQ2d 1245 (BPA & I 1988). The function of the claims is not to specifically exclude possibly inoperative embodiments. *Atlas Powder v. E.I. du Pont de Nemours Co.*, 224 USPQ 409 (Fed. Cir. 1984).

In order to satisfy the requirements of §112, first paragraph, "it is not necessary to embrace in the claims or describe in the specification all possible forms in which the claimed principle may be reduced to practice." *Smith v. Snow*, 294 U.S. 1, 11 (1935). The law does not require an applicant to describe in his specification every conceivable embodiment of the invention. *SRI Int'l v. Matsushita Elec. Corp. of America*, 227 USPQ 577, 586 (Fed. Cir. 1985). Moreover, while working

examples drawn to specific embodiments may be desirable, they are not *required* in order to satisfy enablement under §112. *In re Strahilevitz*, 212 USPQ 561 (CCPA 1982). It is well established that working examples are not necessary when one possessed of knowledge of ordinary skill in the art could practice the invention without the exercise of undue experimentation. *Ex parte Nardi*, 229 USPQ 79 (BPA & I 1986).

"In satisfying the enablement requirement, an application need not teach, and preferably omits, that which is well known in the art." *Staehelin v. Secher*, 24 USPQ2d 1513, 1516 (BPA & I 1992). Enablement under § 112 of the statute is determined from the viewpoint of one of ordinary skill in the art at the time of filing the application for patent; i.e., at the time of constructive reduction to practice. The person of ordinary skill in the art brings with him a knowledge and understanding of the entirety of the prior art up until the date of application. A "patent need not disclose, and preferably omits, that which is well known in the art." *Hybritech, Inc. v. Monoclonal Antibodies, Inc.*, 231 USPQ 81, 94 (Fed. Cir. 1986).

Moreover, the first paragraph of § 112 contains no requirement for a *structural* disclosure - a description entirely in *functional* terms can satisfy the enablement requirement. *Ex parte Butler*, 217 USPQ 290 (USPTO Bd. App. 1982). *See*, also, *In re Donohue*, 193 USPQ 136 (CCPA 1977), *Ex parte Billottet*, 192 USPQ 414 (USPTO Bd. App. 1976). Particularly when details of the *structure* at issue are not a critical aspect of the invention claimed, a detailed description of what would be readily apparent to one of ordinary skill in the art serves no practical purpose. "Non-

critical features of the invention may be supported by a more general disclosure than those at the heart of the invention." *In re Stephens*, 188 USPQ 659, 661 (CCPA 1976).

The presently claimed invention involves what is commonly known as "Fluorescence intensity distribution analysis" or "FIDA," which has been applied to a variety of applications as can be demonstrated by the following literature:

- Haupts et al. (*Journal of Biomolecular Screening, 8* (1): 19-33, Feb. 2003). The authors demonstrate a number of applications of FIDA which discriminates molecules by their specific brightness. Examples for assays based on brightness changes induced by quenching/dequenching of fluorescence, fluorescence energy transfer, and multiple- binding stoichiometry are given for important drug targets such as kineases and proteases.
- Rudiger et al. (*Journal of Biomolecular Screening, 6* (1): 29-37, Feb. 2001). The authors describe homogeneous fluorescence-binding assays that are highly amenable to miniaturization. Binding and displacement experiments are demonstrated for various types of G-protein coupled receptors, such as chemokine, peptide hormone, or small-molecule ligand receptors, demonstrating the broad applicability of FIDA.
- Scheel et al. (*Journal of Biomolecular Screening 6* (1): 11-18, Feb. 2001). The authors describe how FIDA can be used to develop homogeneous, non-radioactive high throughput screening assays for membrane receptors. It is demonstrated that ligand affinity, receptor expression level, and potency of inhibitors can be determined using the epidermal growth factor and beta(2)-adrenergic receptors as model systems. The results demonstrate that FIDA

is an ideal method for membrane receptor assay offering substantial benefits for assay development and high throughput pharmaceutical screening.

- Schaertl et al. (*Journal of Biomolecular Screening 5* (4): 227-38, Aug. 2000). The authors have established a new type of homogeneous immunoassay based on nanoparticles being analyzed using FIDA.
- Kask et al. (*PNAS, vol. 96*, no. 24, 13756-13761, 1999). The authors demonstrate the potential of the FIDA technology by studying the hybridization of 5'-(6-carboxytetra-methylrhodamine)-labeled and non-labeled complementary oligonucleotides and the subsequent cleavage of the DNA hybrids by restriction enzymes.

The aforesaid discussion relating to the written description requirement is useful in demonstrating satisfaction of the enablement requirement, as well. The "spatial brightness function" is one of the keywords of the present main claim. The specification explains that, when calculating the expected (or theoretical) distribution of the number of photon counts, the measurement volume is divided into a number of spatial sections. In the specification (starting at page 15 with "What characteristics of the spatial brightness function ...), two consecutive paragraphs are devoted to describing how the spatial brightness function is characterized. The principle that volumes of a number of spatial sections are calculated as functions of a few parameters of the model is described. Also, the principle that the values of the parameters of the model are selected in such a way that the closest fit between experimental and expected distributions of the number of photon counts is achieved, using inter alia experiments on single dye species, is set forth. Further, application

Example 1 (paragraph on page 21 starting with "As the second preparatory step...") provides a description of how values of volumes of spatial sections and the relative contribution to fluorescence light originating from areas of lower spatial brightness were determined. The determined volumes are presented in application Figure 3, as well. In this example, "a simple model of the optical equipment not accounting for aberrations was taken into use". Earlier in the specification, when introducing a simple model of the optical set-up ("conveniently, a relatively simple ...") it was mentioned, that "it might ... be preferred to use the pinhole dimensions and the convergence angle of the incident laser beam as modeling parameters of the spatial brightness function".

Values of volumes of sections of the measurement volume in a confocal optical set-up depend in particular on the convergence angle of the laser beam used to excite the particles in the measurement volume and the diameter of the confocal pinhole. It is well-known to the person skilled in the art that in each cross-section of a laser beam, the intensity is Gaussian, while the beam radius in the focus decreases with increasing convergence angle. The beam radius as a function of the longitudinal co-ordinate is graphically represented in the following figure 1.

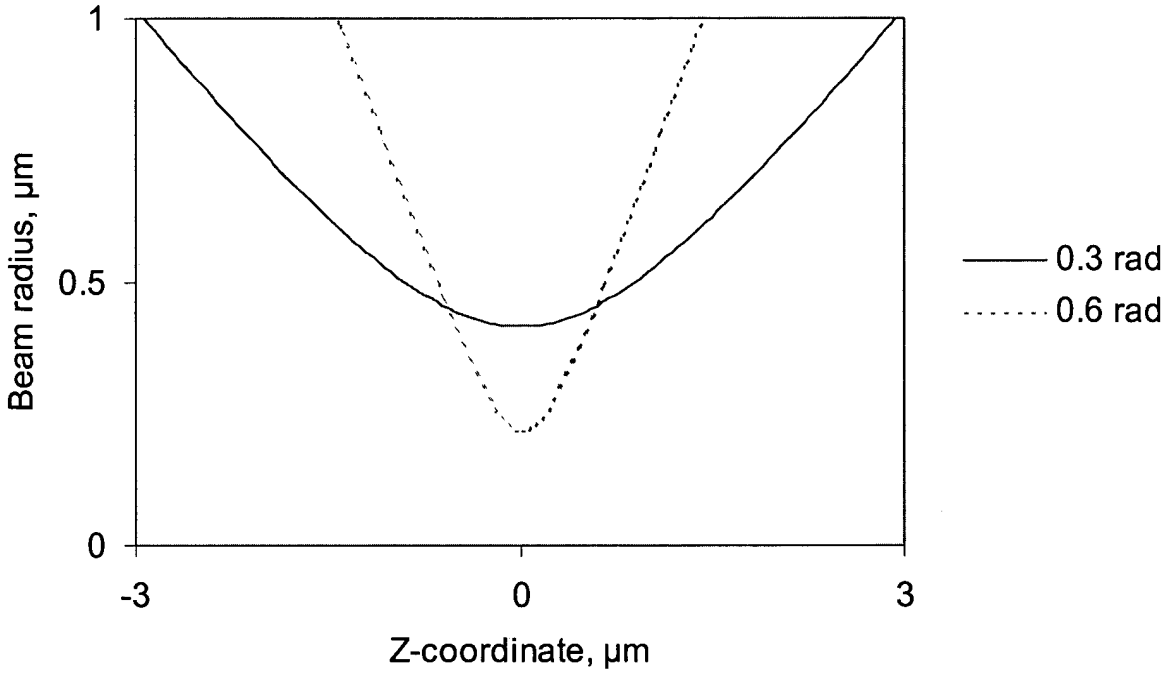

Figure 1. Beam radius depending on the longitudinal distance (z-coordinate) from the focus.

This dependence can be converted into a map of equal intensity lines, as seen on the following Figure 2.

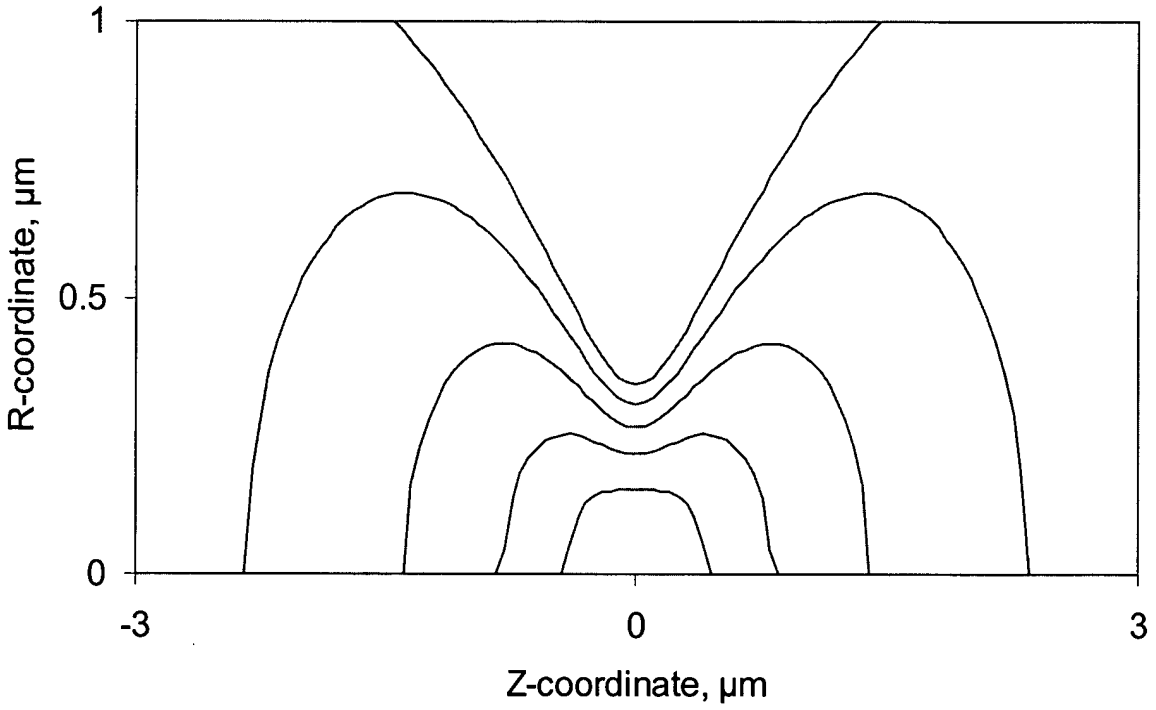

Figure 2. Lines of equal illumination intensity near the focus of the laser beam. The upper graph is calculated for the convergence angle of 0.3 radian, the lower one corresponds to the angle of 0.6 radian. The neighbor lines on the graphs differ by an intensity factor of 1/e.

However, this is not yet the brightness map of a particle. Brightness of particle as detected through a confocal microscope depends not only on intensity of illumination, but also on transmission coefficient that varies with the coordinates of the particle within the measurement volume. The transmission coefficient is the fraction of emission that passes through the pinhole. If a particle is on the focal plane, then its emission is collected into a small spot on the image plane.

If a particle is apart from the focal plane, then its image on the focal plane is a disc which diameter is nearly proportional to the distance of the particle from the focal plane, see the following figure 3.

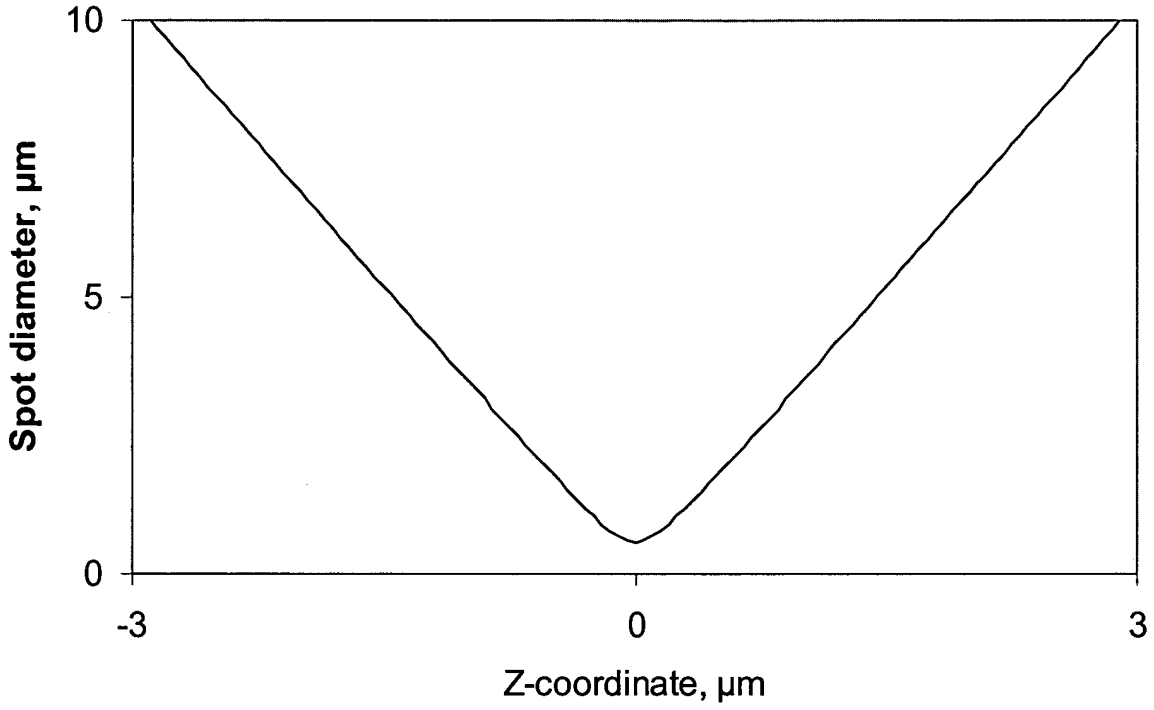

Figure 3. Apparent diameter of the particle versus Z-coordinate. If the particle is not on the focal plane then its apparent diameter increases nearly proportionally with the absolute value of the Z-coordinate.

On basis of the diameter of the image disc and the diameter of the pinhole, the proportion of emitted light passing through the pinhole can be calculated as a function of z- and r-coordinates of the particle. Moving with a particle in r-direction causes a shift of the image in respect of the pinhole. The transmission drops to zero when a particle is so far from the optical axis that the image disc does

not hit the pinhole anymore. The calculated map of the transmission coefficient is presented in the following figure 4 for two different values of the pinhole diameter.

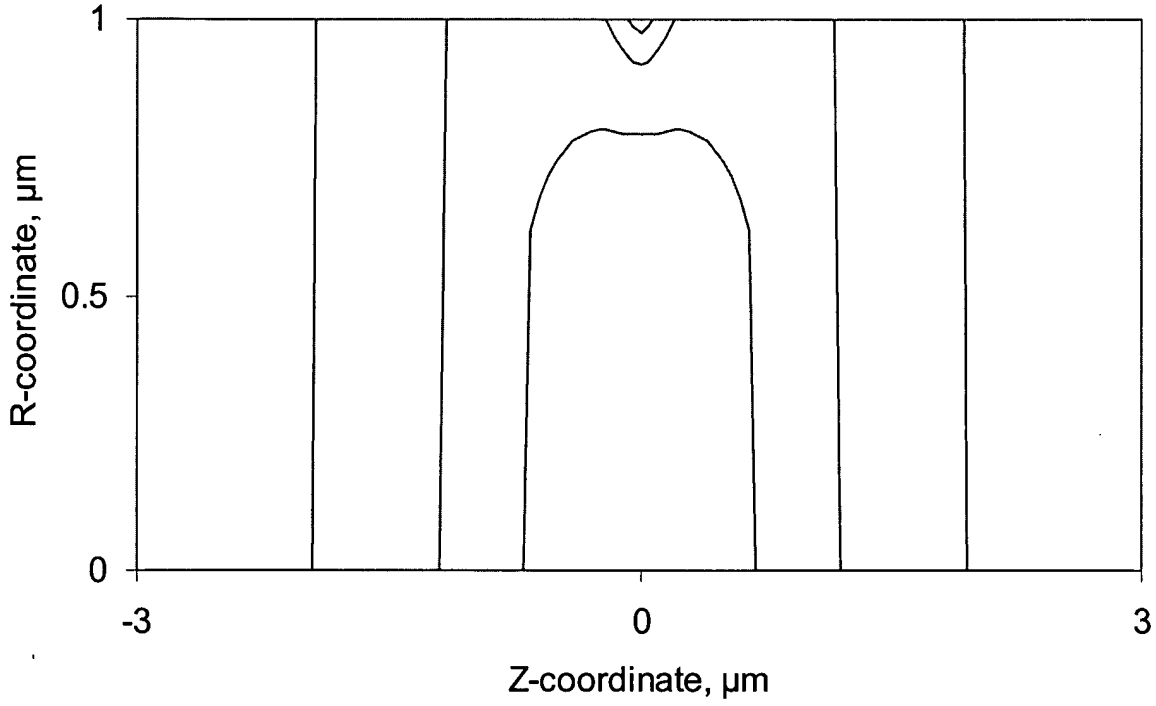

Figure 4. The lines of equal transmission coefficient of a microscope. The upper graph is calculated for the pinhole diameter of 40 :m, the lower one corresponds to the pinhole diameter of 60 :m. The neighbor lines on the graphs differ by an intensity factor of 1/e.

The map of the product of the laser intensity and transmission coefficient is presented in the following figure 5 for two different combinations of the convergence angle and the pinhole diameter.

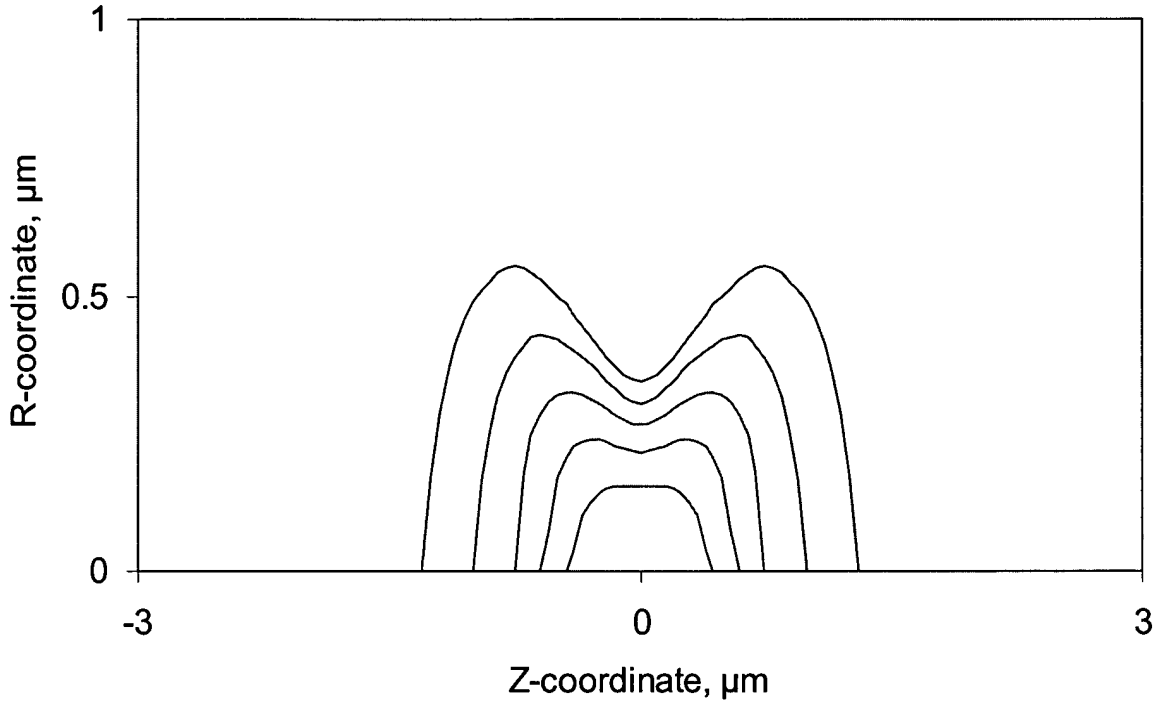

Figure 5. Lines of equal brightness, which is the product of the illumination intensity and the transmission coefficient. The neighbor lines on the graphs differ by a brightness factor of 1/e. The upper graph is calculated for the convergence angle of 0.3 :m and the pinhole diameter of 40 :m, while the lower one corresponds to the convergence angle of 0.6 :m and the pinhole diameter of 60 :m. Sizes of volumes of brightness sections can be numerically calculated from the data graphed here. It is apparent already from the presented graphs that relative sizes of volumes of the brightness sections depend on values of the convergence angle and the pinhole diameter.

The experiment for characterizing the spatial brightness function is preferably done utilizing a single dye solution with a high count rate per particle. The purpose is not to select the true values of the convergence angle and the pinhole diameter in their direct physical meaning. Rather, these

parameters are employed according to the present invention as a means granting a flexibility of the model needed to achieve a good quality of analysis. Thus, convergence angle and pinhole diameter are modeling parameters in the fitting process when their values are determined. This has been expressed in the specification text in particular on pages 15 and 16.

Claims were also rejected under 35 USC 112, ¶2, for allegedly being indefinite. Applicant submits that the changes to claim language represented by the present claims, taken together with the aforesaid discussion, overcomes the rejection.

Favorable action is requested.

Respectfully submitted,

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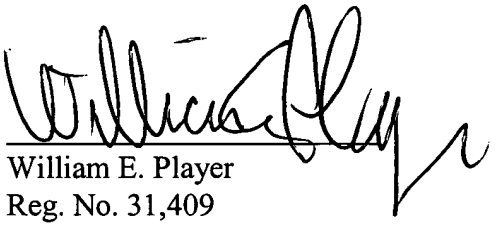
By 

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